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THE MATHERS COCCUS IN THE THROAT IN INFLUENZA

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During February and March, 1922, an epidemic of so-called influenza occurred in Iowa City. The disease was highly contagious, of short duration, and was followed by few complications. A study was made of the bacterial content of the throats of the students of the second year class of the medical school, before, during, and for a considerable time after the epidemic. Cultures were also made from the tonsils and nasopharynx of patients sent to the hospital with influenza and of other students with similar symptoms.

In most cases the symptoms were marked from 3 to 5 days; many of the students remained in bed from 1 to 3 days; others continued to attend classes, although they had some fever and felt rather ill. In a few instances symptoms persisted for 3 weeks. There were three types of symptoms: nasal, laryngeal, and bronchial, all of which were associated with considerable lassitude and general malaise. The general symptoms were mild, however, in the laryngeal form, but the cough was persistent in most cases.

The epidemic began Feb. 18, when 2 students complained of a severe cold, and 42 students of a class of 74 had a similar attack before March 6. There was only an occasional case after this time, until April 5 to 8, when 7 students had a rather severe attack of coryza. Five of these did not have influenza during the first epidemic. Since this time there have been occasional cases of coryza among the students, but the symptoms have been different and the throat cultures have contained a variety of bacteria.

Blood counts were made on 5 students and 6 patients in the hospital with influenza; two students had white counts of 6,200 and 6,800, two hospital patients had similar counts, and one patient had a white count of 12,600. The counts of the others were within the normal range.

In making throat cultures, swabs were passed over the tonsils and posterior pharyngeal wall. The swabs were then washed in tubes containing 1 c c of salt solution. One to three loopfuls of this suspension

Received for publication, June 9, 1922.

were added to melted tubes of agar, which were then poured into plates containing 1 c c of defibrinated sheep blood. After 24 and 48 hours' incubation, an estimate of the number and types of colonies was made. Records were kept of the 4 principal types: (1) Str. hemolyticus; (2) gram-negative cocci; (3) Str. viridans and pneumococcus, and (4) a peculiar green-producing streptococcus. Three colonies of the green-producing streptococci from each plate were streaked to blood agar slants, and tested for bile solubility and the fermentation of inulin. Agglutination tests were then made on the bile soluble strains with 3 types of antipneumococcus serum. Cultures were also made on Loeffler's medium from each student on 2 different occasions and examined for diphtheria bacilli. In the early part of the work cultures were also made on chocolate blood agar to determine the presence of B. influenzae.

TABLE 1
HEMOLYTIC STREPLOCOCCI FOUND IN THROATS OF STUDENTS

Time of Cultures	Total Cultures	Students Cultured	Cultures Containing Str. Hemolyticus	
Before attack During attack After attack Those not affected	23	17	8 (34%)	
	68	45	10 (15%)	
	104	55	33 (31%)	
	35	14	8 (22%)	

Hemolytic streptococci were found one or more times in the throat of 33 students, and in 10 they were present at every examination. The number of hemolytic streptococci in the throat was often increased after the disease but in some instances the number was less during the acute stage. In 3 students they were found in small numbers before the attack, were absent during the attack, and were the predominant organism following the disease. From table 1 it appears that the number of these organisms in the throat was less during the acute stage of the disease than at other times. The number of cultures taken from the class of students with the number of times hemolytic streptococci were found are given in table 1.

Diphtheria bacilli were not found in two cultures taken from each student.

The influenza bacillus was isolated 8 times in 23 cultures from normal throats and 3 times in 19 cultures during the acute stage of the disease. The cultures taken during the acute stage were overgrown

with a green-producing streptococcus. The attempt to isolate the influenza bacillus was discontinued in the latter part of the work.

Staphylococci and gram-negative cocci were found in larger numbers in normal throats. In many cases none of these organisms was found during the acute stage in dilutions when there were isolated colonies on the plates. In a few cases gram-negative cocci were the predominant organisms after the attack.

Although no particular attempt was made to isolate the pneumococcus from each case, in those cases in which there were different types of green colonies, at least one of each kind was transferred to blood-agar slants. All strains which were soluble in bile were then typed with the antipneumococcus serums. Pneumococci were isolated 19 times from 15 students. Type 1 was found 3 times; twice from the same student at different examinations. Type 2 was found once and type 4, 15 times. Type 4 was isolated from 3 cases at 2 different examinations. The pneumococci were isolated more often from cases of slight pharyngitis occurring after the epidemic.

Direct smears and cultures on blood agar gave a peculiar green-producing streptococcus as the predominating organism during the first few days of the disease; it was isolated also from the conjunctival secretions in 2 cases with marked conjunctivitis. This streptococcus appears to be the same as that described by Mathers, and more recently by others, in connection with their work on influenza. A review and discussion of the literature concerning this streptococcus has been made by Tunnicliff. This streptococcus has been found by Pilot and Pearlman in 16% of extirpated adenoids.

On the surface of blood agar the organism produces large, flat, moist, greenish colonies, with a tendency to coalesce. In the depth of the medium the colonies are larger than the common Str. viridans. The central colony has an indefinite outline, but this is surrounded by a definite brownish-green zone, and surrounding this there is a narrow hemolytic zone. The hemolytic zone usually becomes definite in 48 hours. The morphology of this organism is much like that of the pneumococcus. In strains from the sputum, milk, and inulin serum

¹ Tunnicliff: Jour. Am. Med. Assn., 1918, 71, p. 1733.

² MacDonald: Brit. Med. Jour., 1919, 2, p. 481. Rosenow: Jour. Am. Med. Assn., 1919, 72, p. 31 and 1604. Jordan: Jour. Infect. Dis., 1919, 25, p. 28. Abstracts of foreign literature compiled by the British Research Committee in the Jour. Am. Med. Assn., 1918, 71, p. 1573.

³ Jour. Infect Dis., 1920, 26, p. 405.

⁴ Ibid., 1921, 29, p. 51.

water, a capsule could usually be seen. It was often difficult to find them in inulin serum water in which acid and coagulation had developed. Quite often a short chain was found in which only one or two cocci were stained. It was practically impossible to find them in coagulated inulin serum water after 5 days' incubation. The relation of this streptococcus to influenza is given in table 2. Only those plates on which there were between 50 and 500 colonies are included in this table.

The cultures taken 24 hours before were taken as controls and 3 of the persons with practically pure cultures at this time remained in bed the next day. All of the others complained of definite symptoms the following day, although they were more mild in those with few of these organisms in the throat. Among the 6 who did not have these cocci during the acute stage, there were 2 in which the hemolytic strepto-

TABLE 2
RELATION OF STREPTOCOCCUS TO INFLUENZA

	24 Hours Before Illness	1st and 2d Days of Illness	5-7 Days of Illness	4 Weeks or More After Illness	Those Not Affected or 5 Days Before Illness
In practically pure culture Predominant organism Few None	5	22	4	0	5
	1	14	5	2	2
	2	3	2	16	6
	2	6	4	58	12

cocci were so numerous that other types could not be isolated. In another case a gram-negative coccus was the predominant organism. The majority of the colonies which developed in the remaining 3 were green-producing streptococci whose morphology and cultural characters were different. There were also many colonies of gram-negative cocci on 2 of these.

The fermentation reactions were tested of 2 typical colonies isolated from each of 34 persons, most of them from acute cases among the students, although representative strains from different stages of the disease—from those not affected and from influenza patients in the hospital—were included. The ability to ferment carbohydrates was tested in broth containing 1% of the sugar and 1% of Andrade indicator. Readings were made after 2 and 5 days' incubation. Dextrose, levulose, maltose, sucrose, and lactose were uniformly fermented; 3 strains, however, failed to ferment lactose. The majority of the cocci produced acid in inulin, salicin, and raffinose. Of 209 strains tested in inulin serum water, 173 produced acid. Mannite and dextrin were not

fermented. Milinska,⁵ working with apparently the same organism, found a few strains that did not ferment lactose, and some strains which fermented mannite. By their fermentation reactions he divides these cocci into 4 types.

The virulence of 8 strains for rabbits and mice was tested by intraperitoneal injections of 0.5 to 1 c c of 24-hour broth cultures into rabbits, and 0.1 to 0.3 c c into mice. All of the animals survived these injections. A rabbit injected with the growth from 2 blood agar slants died in 40 hours. Two rabbits injected with 0.5 c c of sputum from severe cases died in 48 hours. This streptococcus was isolated from the heart blood in both cases, but in one a type 4 pneumococcus was the predominant organism. Three of 4 mice injected with small amounts of sputum died in 24 hours. One of these was injected with the same sputum that was injected into the rabbit from which type 4 pneumococcus was isolated. The pneumococcus was the only organism isolated from the mouse. The streptococci were found in pure culture in the other mice. From these experiments it appears that the cocci are not highly virulent for mice and rabbits.

CONCLUSIONS

Hemolytic streptococci were present in 47% of the students examined 2 or more times, and appeared in smaller numbers during an attack of influenza. The pneumococcus was isolated from 15 students, and type 4 was found in 12 of these. The influenza bacillus could be isolated more often from the normal throat than from the throats of influenza patients. A green-producing streptococcus resembling the pneumococcus in morphology but insoluble in bile was found to be the predominating organism in an influenza epidemic affecting 75% of a class of students. The same organism was found as the predominant one in the throats of other students with similar symptoms and in the throats of patients in the hospital with influenza. This coccus grows characteristically on blood agar and uniformly ferments dextrose, levulose, maltose, sucrose, and lactose. In most cases inulin, salicin, and raffinose are also fermented. Dextrin and mannite are not fermented. It is only slightly virulent for mice and rabbits. The relation of this organism to the epidemic is very interesting. For convenience of discussion and because of the early study by Mathers of this organism in relation to influenza, the name of Streptococcus mathersi is suggested for it.

⁵ Przeglad Epidemiol., 1920, 1, p. 29; abst., Offic. Inter. d'Hygiene Publique, 1922, 14,